University of Nevada, Reno

Assessment of Biomarkers of Intrauterine inflammation induced preterm labor

A thesis submitted in partial fulfillment
of the requirements for the degree of

Bachelor of Science, Biology and the Honors Program

by

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May, 2015

UNIVERSITY OF NEVADA RENO

THE HONORS PROGRAM

We recommend that the thesis
prepared under our supervision by

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entitled

Assessment of Biomarkers of Intrauterine inflammation induced preterm labor

be accepted in partial fulfillment of the
requirements for the degree of

BACHELOR OF SCIENCE, BIOLOGY

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May, 2015

Abstract

Preterm labor, classified as birth before 37 weeks, is the manifestation of uterine contractions with sufficient frequency and intensity to cause progressive cervical thinning and dilation of the cervix prior to term gestation. Preterm labor is a consequence of a complex cluster of complications with many overlapping factors. Amongst this cluster of problems, intrauterine infection and inflammation pathway is widely studied as a source of preterm labor (Johnson et al., 2014; Viscardi et al., 2004; Tency, 2014). Intrauterine infection can be described as the presence of microorganisms in the amniotic cavity. The chemical mediators that are released as a result of an immune response to infection include cytokines and chemokines, prostaglandins and matrix-degrading enzymes. These chemical mediators are proposed as possible biomarkers of intrauterine infection (Challis et al., 2002). Biomarkers are objective indicators of a certain medical state that can be measured using various laboratory procedures. The identification of biomarkers for intrauterine infection can help predict clinical outcome as well as direct treatment methods to preempt potential preterm labor (Strimbu and Tavel, 2011). Numerous research studies including Kusanovic (2010), Tency (2014), Amalinei (2007), Romero (1990), and Bernal (1987), have found increases in sTREM-1, MMP, cytokine, and prostaglandin concentrations during intrauterine infection. Currently there are various antibiotic regimens that have been shown to have significant effect in preventing the progression of bacterial vaginosis, in pregnant women. Bacterial vaginosis is the existence of infectious bacteria in the vagina. Normally, this mild infection in the vagina will disappear; however, in if it does not, bacterial vaginosis will progress to the advanced state of intrauterine infection, termed chorioamnionitis. Thus, these antibiotic regimens are not effective in treating the pathological state, of intrauterine infection and chroioamnioitis (Ovalle et al., 2006; Hutzal et al., 2008). However, recent findings regarding

the heterologous etiology of preterm labor have promise for the development of an effective treatment. Using reliable biomarkers in conjunction with targeted therapy towards known contributors to preterm labor may result in a decrease in the occurrence of preterm labor and increase the health of newborns.

Acknowledgements

This research paper is made possible through the help and support from everyone, including: parents, teachers, family, friends, and in essence, all sentient beings. Especially, please allow me to dedicate my acknowledgment of gratitude toward the following significant advisors and contributors:

First and foremost, I wish to express my deepest appreciation to my thesis advisor, Dr. Amy Altick. Without her guidance, expertise, encouragement, and persistent help, this thesis would not have been possible. I am extremely grateful and indebted to her for all her support.

Secondly, I would like to gratefully acknowledge the support, aid and recommendations for research from Dr. Tamara Valentine, my professor and director for the Honors Program, during the past year.

Thirdly, I would like to sincerely thank Dr. Heather Burkin for her willingness to extend her recommendations and expertise for my research paper.

Finally, I take this opportunity to record my sincere thanks to all the faculty members of the Honors Program at the University of Nevada, Reno for their help an encouragement.

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1. Introduction to intrauterine infection induced preterm labor

Preterm labor, classified as birth before 37 weeks, is a continual challenge in prenatal healthcare (World Health Organization, 2015). In 2012, 11.11% of the infants born in the United States were premature, and 35% of all infant deaths in 2010 were due to preterm-related complications (Division of Reproductive Health and Centers for Disease Control, 2014). Amongst the various demographical factors, racial, ethnic and socioeconomic disparities have the most significant correlation to rates of preterm labor. In 2003, the highest occurrence of preterm labor was within the non-Hispanic African Americans at 17.8%, while the lowest rates were in Asian and Pacific Islander women at 10.5%. The same study found a preterm labor rate of 11.5% for Caucasian women (Behrman and Butler, 2007). Major organs such as the lungs, heart, and brain of infants require the final weeks of gestation to fully develop. Thus, infants that survive preterm labor are at a higher risk of developing significant health consequences such as cerebral palsy, mental retardation, respiratory problems, and hearing and vision impairment (Flood and Malone, 2012). In addition to the complications to the mother and infant, preterm labor is a major social impact on health insurance costs, education and social services. The annual cost of preterm labor is around $26 billion in 2004 (CDC/National Center for Health Statistics, 2004). The $26 billion spent in 2004 was merely for intensive care unit costs; preterm labor does not include the costs necessary for long-term care for infants who survive but are affected by preterm labor.

Preterm labor is a consequence of a complex set of factors seemingly originating in the mother. This set of complications or causes can be separated into individual biological pathways. A biological pathway is described as a series of interactions between molecules in a cell that

ultimately lead to a certain product or a change in the cell. The first pathway pertaining to the study of preterm labor is the activation of maternal/fetal hypothalamic-pituitary-adrenal (HPA) axis under the effects of stress. The second pathway is pathologic uterine distension resulting from excessive stretching of the uterus. Ischemia decidual hemorrhage, the third pathway, is the result of blood clot formation. The final pathway, infection and inflammation, is characterized as the initiation of a proinflammatory cascade in response to intrauterine infection.

Although the four biological pathway known to lead to preterm labor have their own unique upstream initiators, they all share common downstream effectors of preterm contractions (Galinsky et al., 2013). For example, matrix metalloproteinases (MMPs), enzymes capable of degrading extracellular matrix proteins of uterine tissues, play a key role in initiating preterm labor. MMPs can initiate preterm labor correlated to infection, uterine over-distension, or preterm premature rupture of membranes (Behrman and Butler, 2007). Any of these complications can activate the common pathway leading to parturition, which is classified as "preterm" labor if initiated before 37 weeks of gestation. The common pathway of normal parturition involves anatomic, biochemical, immunological, endocrinological, and clinical events occurring in the mother and the fetus (Romero et al., 2003). This pathway is shared in both preterm and term labor. The chief difference between preterm and term labor is that term labor is the result of activation of components within the common pathway anytime after 37 weeks of gestation, while preterm labor (<37 weeks) is the result of pathological activation of the common pathway prematurely (Behrman and Butler, 2007).

Intrauterine infection and the inflammatory pathway, which is the focus of this paper, are widely studied as a source of preterm labor (Johnson et al., 2014; Viscardi et al., 2004; Tency, 2014). Intrauterine infection can be described as the presence of microorganisms in the amniotic

cavity. Bacterial pathogens can gain access to the amniotic cavity and fetus through multiple pathways (Romero et al., 2003). As a part of the immune system, the body's attempt to self-protect following an infection of bacterial pathogens is to release various chemical mediators. The chemical mediators that are released as a result of an immune response include cytokines and chemokines, which can affect the activity of prostaglandins and matrix-degrading enzymes. These chemical mediators are classified as biomarkers of intrauterine infection because they signal the presence of an infection (Challis et al., 2002). The identification of biomarkers can help with the prediction of clinical outcomes such as the premature cervical ripening and rupture of membranes. This in turn can help design appropriate treatment methods for preterm including effective antibiotic regimens (Strimbu and Tavel, 2011). The identification of specific biomarkers of intrauterine infection helps define the infection and inflammation biological pathway, which enables researchers to be a step closer to understanding the cause of preterm labor.

Intrauterine infection and inflammations are known to trigger early uterine contractions, membrane rupturing and cervical ripening (Hay, 2002), and subsequently premature parturition. Intrauterine inflammation commonly presents as chorioamnionitis. Chorioamnionitis is the inflammation of the chorion, amnion, and placenta (Galinsky et al., 2013), and it can be either subclinical or clinical, both of which can lead to preterm labor if left untreated. Intrauterine infection and inflammation is highly associated with preterm labor as 40% of all preterm labor have a positive amniotic fluid and/or chorioamniotic-space bacterial culture, indicating an intrauterine infection. Significantly, the majority of intrauterine infection related preterm labor cases were associated with subclinical chorioamnionitis (Romero et al., 2003).

In contrast to clinical chorioamnionitis, which exhibits signs of high maternal fever, leukocytosis, tachycardia, uterine tenderness, and preterm rupture of membranes (Hagberg et al., 2002), subclinical chorioamnionitis is an asymptomatic. Chorioamnionitis is classified histologically by inflammation of the chorion, amnion, and placenta (Viscardi et al., 2004). To diagnose subclinical chorioamnionitis, either an amniotic fluid culture must be obtained by amniocentesis before delivery, or a histopathologic examiniation of both the placenta and umbilical cord must be performed after a woman has delivered prematurely, which is too late to prevent preterm delivery and mitigate the consequences for both the mother and infant. When detected, both subclinical and clinical chorioamnionitis can be treated or at the very minimum contained with the use of certain antibiotics. Clinical chorioamnionitis can be diagnosed and treated with the use of antibiotics more successfully compared to subclinical chorioamnionitis (Johnson et al., 2014). Due to the extensive and invasive procedures that are required for diagnosis of subclinical chorioamnionitis, women that may be at risk for preterm labor and delivery due to subclinical chorioamnionitis go undetected.

In this research paper, I will define the process and role of the inflammatory response related to subclinical chorioamnionitis and preterm labor. I will then discuss the progress made towards identifying credible biomarkers of activation of maternal/fetal HPA axis under the effects of stress, uterine overdistension, uteroplacental thrombosis and decidual hemorrhage, and systemic and intrauterine infection and chorioamnionitis. Finally, I will assess whether early detection of these biomarkers can aid in the containment of preterm labor.

2. Biological pathways to spontaneous preterm parturition

Understanding the process of preterm labor remains one of the most important challenges in obstetrics. There are various known contributors to preterm labor including (Galinksy et al., 2013). However, an exact mechanism for the initiation of preterm labor, prevention, or treatment of preterm labor is yet to be determined. Understanding the underlying pathophysiology of preterm labor lies within the study of biological pathways to spontaneous preterm parturition.

Preterm labor is the result of multifactorial processes varying according to gestational age, genetics, and environmental factors. As mentioned previously, these multifactorial processes are categorized into four pathways: activation of maternal/fetal HPA axis under the effects of stress, uterine overdistension, uteroplacental thrombosis and decidual hemorrhage, and systemic and intrauterine infection. Gene-environment interactions and familial and intergenerational influences have a strong predictive value in determining the initiation and progression of one or more of these pathways (Behrman and Butler, 2007). Interventions for the prevention of preterm labor can either be directed towards the inhibition of a specific upstream initiator of a given pathway or the inhibition of the shared downstream effectors of all the pathways (Gravett et al., 2010). Subsequently, this understanding is important in the development of effective diagnostic criteria. Below, the four pathways to preterm labor are discussed in detail with an emphasis on the intrauterine infection and inflammation biological pathway.

2.1 Activation of the maternal-fetal hypothalamic pituitary–adrenal (HPA) Axis biological pathway

HPA axis, our body's central stress response system, becomes activated as a result of fetal maturation. In the case of stress, the HPA axis is prematurely activated and drives a cascade

of events starting with the activation and expression of an increased level of placental corticotropin releasing hormone (CRH). Subsequently, the expression of placental CRH stimulates fetal secretion of cortisol and DHEA-S and placental release of estriol and prostaglandins. Ultimately, the premature expression of these molecules leads to preterm labor through cervical ripening and membrane structure. It is known that women delivering preterm have significantly elevated CRH levels in comparison to women delivering at term (Hobel et al. 1999). However, as a result of the multiple factors such as infection that contribute to premature HPA axis induced preterm labor, CRH is not currently used as a biomarker (Behrman and Butler, 2007).

2.2. Pathologic uterine distension biological pathway

Activation of pathologic uterine distension, is recognized to play a role on the onset of preterm labor associated with multiple gestations, excessive accumulation of amniotic fluid, and excessive birth weight of newborn (Loudon et al., 2004). The intrauterine pressure required to distend the uterine cavity remains relatively constant throughout the entirety of the gestation cycle. Progesterone and endogenous myometrial "relaxing agents" such as nitrogen oxide play a role in the maintenance of a relative constant intrauterine pressure. Excessive uterine distention results in the increase of myometrial contractility, upregulation of oxytocin receptors, prostaglandin release and expression of gap junction proteins (Koucký et al., 2009). These effects can occur alone or in combination with one another to initiate preterm labor through preterm rupture membranes and preterm cervical effacement and dilation without labor. Uterine distension resulting in preterm labor is diagnosed through the use of diagnostic methods such as ultrasound and radiological examinations during regular prenatal checkups. Therefore, the use of

biomarkers are not needed, rather the current focus is on relieving overdistension and controlling the effects of eventual preterm parturition (Galinksy et al., 2013).

2.3 Uteroplacental thrombosis and decidual hemorrhage biological pathway

During the uteroplacental thrombosis and decidual hemorrhage pathway, an enhanced production of thrombin and high levels of fibrin deposition provoke uteroplacental thrombosis. Uteroplacental thrombosis is the formation of a blood clot resulting from leukocytic and thrombin infiltration of the vascular wall. In vitro, thrombin has shown to significantly increase the levels of matrix metalloproteinases (MMPs), enzymes capable of degrading the extracellular matrix, in decidual cells and fetal membranes (MacKenzie et al., 2004). This increase in MMPs activates preterm premature rupture of membranes (PPROM) and decidual hemorrhage. Further, thrombin triggers a dose-dependent increase in decidual IL-8. IL-8 is a cytokine responsible for the infiltration of neutrophils, a rich source of MMPs (Behrman and Butler, 2007). Taken together; the increased production of MMPs as a result of uteroplacental thrombosis and decidual hemorrhage pathway can be used as a potential biomarker for preterm labor.

2.4 Intrauterine infection and inflammation biological pathway

Infection and inflammation pathway initiates a proinflammatory cytokine-prostaglandin cascade that influences early uterine contractions, membrane rupturing, and cervical ripening. Infections have a strong correlation with preterm labor with at least 40% of preterm births being correlated to intrauterine infection (Goldenberg et al., 2008; Romero et al., 2003; Agrawal and Hirsch, 2012). Apart from intrauterine infection, other common sources of infection relating to

preterm labor include lower genital tract infections, systemic maternal infections, asymptomatic bacteruria, and maternal periodontitis (Behrman and Butler, 2007). The focus of recent study has been on intrauterine infections as they are the most prominent and potentially preventable causes of preterm birth (Romero et al., 2002; Agrawal et al., 2012; Behrman and Butler, 2007). One reason intrauterine infections are classified as the most prominent is due to the belief that they are responsible for up to 50% of extreme preterm births occurring at 28 weeks or less of the gestation cycle. Preterm births occurring this early within the gestation cycle are dangerous for the fetus with the higher probability of neonatal mortality and morbidity (Gravett et al., 2010).

Intrauterine infections are caused by existence of infectious microorganisms in the uterus. Microorganisms can gain access to the amniotic cavity and the fetus by ascending from the vagina and the cervix, being transported by blood, dissemination through the placenta, retrograde seeding from the peritoneal cavity through the fallopian tubes, and accidental introduction at the time of invasive procedures. Of these pathways, ascending from the vagina and the cervix is the most common route that leads to intrauterine infection (Steer et al., 2011). Ascending infections are comprised of five stages. The first stage is classified by the overgrowth of facultative organisms or a change in the microbial flora with the existence of pathologic organisms in the vagina, cervix, or both. As mentioned previously, this existence of infectious organisms in the vagina is termed bacterial vaginosis. The existence of microorganisms in the vagina and/or the cervix enables those microorganisms to gain access to the intrauterine cavity through restricted entrances such as the endocervical canal, the second stage of infection. With the gain of access into the intrauterine cavity, a localized inflammatory response is produced causing local chorioamnionitis, which is the third stage. Thereafter, during stage four, the microbial infection may invade the fetal vessels (choriovasculitis) or proceed through the amnion (amnionitis) into

the amniotic fluid. The invasion of the fetal vessels and the progression through the amnion result in a microbial invasion of the amniotic cavity, which is the fifth and the final stage of this process, classified as fetal infection (Berghella, 2010).

Most intrauterine microbial colonization is subclinical, meaning that microbial colonization is undetectable without the analysis of the amniotic fluid. Thus, the common methods that are used for identification purposes are standard microbiologic methods such as cultivation techniques. Collection of amniotic fluid samples through amniocentesis and histopathologic examinations are carried out by obtaining a specimen of the amniotic fluid, followed by the cultivation of that particular specimen to determine the existence of bacteria or microorganisms in the amniotic cavity. It is important to note that a negative culture does not necessarily indicate the absence of bacteria in the amniotic cavity. A negative culture may be the result of poor growth conditions provided by the laboratory (Berghella, 2010). The conclusion that the occurrence of preterm labor may be the primary consequence of microbial activity or invasion in the amniotic fluid is demonstrated by the high percentage of microbial flora present in women who deliver prematurely within 23-26 weeks of gestation (45%) compared to 11% of microbial flora that is present in women who deliver between 31-34 weeks (Gravett et al., 2010). Taken together, evidence obtained through histological examinations concludes that a subclinical infestation of infectious microbial activity in the uterus is highly correlated to preterm labor. Thus, the understanding and identification of biomarkers of the inflammatory response to infection is a promising approach to preventing a majority of intrauterine induced preterm births.

3. Animal Models of Chorioamnionitis and Intrauterine Inflammation

The study of human parturition is heavily based on animal models as a result of the conscientious limitations and ethical considerations associated with the use of humans in

controlled studies. Each animal model contains its own advantages and disadvantages. Overall, the use of animal models is beneficial in helping define the physiology of maintenance and termination pregnancy. In addition, researchers are able to explore the normal time-sensitive mechanisms of parturition in comparison to the consequences of infection to elucidate pathways leading to preterm birth.

One method of producing intrauterine inflammation in animals such as rats and rabbits is by exposing the fetus to lipopolysaccharide (LPS). Lipopolysaccharides are macromolecules that are major components of the outer membrane of Gram-negative bacteria. In the absence of intrauterine infection, lipopolysaccharides have the capacity to induce an inflammatory cascade. This downstream cascade of inflammatory response stimulates macrophages that in turn lead to the production of various cytokines similar to the same process observed with intrauterine infection in pregnant women at preterm delivery (Grigsby et al., 2002). Thus, the administration of lipopolysaccharides to pregnant animal models such as rabbits, sheep and rats can be used to model chorioamnionitis.

Lipopolysaccharides have been introduced to animal models such as rats and rabbits through intravenous and intraperitoneal administration. Intravenous studies, characterized as the administration of LPS through veins mimicking the blood born pathway of infection, have shown to increase uterine contractility, systemic and placental inflammation, and preterm delivery (Feng et al., 2010). Intraperitoneal studies, characterized by the administration of LPS into the peritoneum (body cavity) to mimic the retrograde route of infection, have shown similar effects to that of intravenous administration, however to a lesser extent. Most severe forms of clinical chorioamnionitis were induced by injecting LPS into the cervix, mimicking the ascending pathological development of intrauterine infection. The injection of LPS into the cervix can lead

to high-grade placental inflammation which is associated with a maternal systemic inflammatory response extending to the fetus (Galinsky et al., 2013).

As an example of using animal models to study basic mechanism of infection induced preterm labor, the inflammatory state can be induced in sheep by administrating LPS maternal side of the placenta. . In one particular study, the injected LPS was used at doses higher than those used systemically to induce an inflammatory state. This experimental design led to the discovery of markedly elevated fetal cortisol and prostaglandin E_2 (PGE_2) concentrations in the amniotic fluid. The exact mechanism(s) that initiate the increased production of PGE_2 was unknown. However, it has was proposed that maternal LPS administration may stimulate the endometrium to secrete cytokines, which then induce the placenta to secrete high concentrations of prostaglandins (Grigsby et al., 2002). Later work showed that the increase in PGE_2 production is part of chain reaction that culminates in preterm labor. The chain reaction starts with the generation of an increased amount of arachidonic acid released by phospholipase A_2 of infectious bacteria, which leads to the production of an increased amount of prostaglandin, eventually leading to preterm labor (Han et al., 2004).

In studies using nonhuman primate models such as rhesus monkeys, the animals were directly infected with an intra-amniotic inoculation of group B *Streptococcus* to determine the change in concentration of cytokines and prostaglandins in the amniotic fluid. Group B *Streptococci* are organisms correlated to preterm birth. In this model, it was demonstrated that interleukin-6 (IL-6), tumor necrosis factor-α, interleukin-1β in parallel with increases in amniotic fluid prostaglandin E2 and prostaglandin F2α are elevated during amnionic infection (Gravett et al., 1994). The production of these chemical mediators occurred prior to an increase in uterine

contractility and any clinical signs of infection. Thus, it was concluded that their exists a cause-and-effect relationship between intra-amniotic infection and preterm labor.

Collectively, these animal studies provide evidence supporting the existence that an inflammatory response can be induced by lipopolysaccharides and inoculations of various pathogens. The inflammatory response leads to the production of higher concentrations of pro-inflammatory cytokines and subsequently, preterm labor. However, it is important to recognize the difference in relevancy in this process between animal models and human parturition. Sheep have been one of the most relevant and valuable models for human preterm birth. The gestational period for sheep is similar to that of humans'. Both humans and sheep produce a small number of offspring during their lifetime, with typically one or two fetuses per gestation (Ratajczak et al., 2010). The use of mice provides another good model for the study of human parturition. One particular reason for the use of mice that has proven useful is the ability to manipulate their genome. Researchers are able to target particular genes with the potential to influence parturition and target those genes for their studies. In addition, both mice and humans share several components of the cascade of events occurring during parturition. Some of these conserved components include prostaglandins that serve a role in uterine contractile and contraction-associated proteins that activate the myometrium. Thus, these animal models are used to elucidate the role of cytokines and inflammation in human preterm births associated with infection. This review of animal models for understanding the physiology of parturition can further the understanding of fundamental mechanisms and predictors of human preterm labor, develop more effective therapeutic agents, and promote treatment measures to prevent the high incidence of preterm labor deliveries.

4. Inflammatory Response to Infection

The existence of intrauterine microbial activity of a pregnant mother can lead to an infection followed by the activation of an inflammatory response. The pathophysiology of infection-associated preterm labor differs from term labor in that infection-associated preterm labor leads to the production of elevated levels of proinflammatory cytokines, leading to the inflammatory response cascade that play a central role in the pathogenesis of infection-associated preterm labor (Behrman et al., 2007). Upon an encounter of a pathogen, the human body's first line of defense involves physical and chemical barriers including mucous membranes, hairs and cilia, gastric juice, tears, and skin. Epithelial surfaces composed of tight junctions binding individual cells together provide the strongest barrier against invasion of pathogens. This first line of defense also includes cellular defenses such as active phagocytes. These phagocytes are non-specific and engulf any foreign pathogen that enters the body. If this first line of defense is unable to prevent the entry of infectious bacteria or foreign pathogen, the immune system initiates an adaptive immune response, which activates a cascade of events involving pathogen recognition receptors. These pathogen recognition receptors bind to specific molecular structures on the surface of the pathogens, this binding in turn, attracts leukocytes such as neutrophils and macrophages. Once these leukocytes recognize receptors bound to pathogens, they engulf the pathogens leading to a proinflammatory cytokine-prostaglandin cascade. This proinflammatory cytokine-prostaglandin cascade induced by microbial infection results in the production of effector molecules such as cytokines including interleukin 1 (IL-1) and tumor necrosis factor (TNF-α), chemokines, prostaglandins, proteases and other enzymes (Agrawal and Hirsch, 2012). During the pathophysiology of infection-associated preterm labor, elevated levels of the effector proinflammatory cytokine and prostaglandins are produced. Once these effector mediators are produced, they then lead to the production of a coordinated response involving uterine

contractions, placental detachment, infiltration of inflammatory cells into gestation tissues, and a series of biochemical and structural changes in the cervix leading to ripening and weakening of the fetal membranes (Agrawal and Hirsch, 2012). Due to the chronic and subclinical nature of most intrauterine infections, biochemical and structural changes in the cervix may not show clinical signs of preterm labor. Even if chronic and chorioamnionitis do exhibit clinical signs, it is likely that these cascades have been activated well before the onset of preterm labor, thus precluding antibiotic therapy treatments from being effective (Strimbu and Tavel, 2010).

While preterm labor can be a result of intrauterine infection coupled with the expression and activation of an immune response, it is critical to recognize that the process of normal human parturition at term also involves a similar immune response pathway with the production of some of the same chemical mediators. Thus, human parturition, whether it is at term or preterm, is classified by an inflammatory response in the cervix, myometrium, and choriodecidua. Various studies that have analyzed the gene expression of these tissues during labor at term have provided evidence indicating the expression of a cluster of proteins resembling an inflammatory signature (Haddad et al., 2006). The reason behind the existence of an inflammatory response is the strategic placement of the choriodecidua that creates conditions for fetal antigenic exposure to the maternal immune system (Haddad et al., 2006). Nutrients and waste products are exchanged across the placenta. The placenta is constructed of syncytiotrophoblast layers of highly vascularized fetal villi that directly project into the placental pool of maternal blood (Phillips et al., 2014). Maternal and fetal tissues and blood are most often allogeneic. Due to this genetic dissimilarity, the maternal system must carry out a local immune response to provide tolerance of the fetal semi-allograft for a successful gestation. Most of the same placental and chemical markers expressed by these tissues play a role during both term and preterm parturition

(Phillips et al., 2014). Examples of such markers are Triggering receptor expressed on myeloid cells (TREM), matrix metalloproteinases, certain cytokines and prostaglandins. Thus, the key to distinguishing labor at term vs. labor at preterm is the examination of the difference in the concentration levels of the expressed chemical mediators.

5. Biomarkers of intrauterine infection and inflammation

Biomarkers are objective indicators of a certain medical state that can be measured using various laboratory procedures. Infection in the amniotic cavity evokes an immune response involving the pathological release of sTREM-1, cytokines, chemokines and prostaglandins together with the production of matrix-degrading enzymes. Therefore, these proteins have the potential to serve as biomarkers that can indicate the presence of subclinical intrauterine infection in pregnant women. Early detection of intrauterine infection can aid in the development of effective treatment methods against the initiation of preterm labor. Thus, it is important to identify biomarkers of intrauterine infection and inflammation for understanding and preventing preterm labor.

5.1 sTREM-1 concentrations

Triggering receptor expressed on myeloid cells (TREM) proteins are transmembrane glycoproteins belonging to the immunoglobulin superfamily receptors. TREM are mainly expressed on monocytes and neutrophils as cell surface receptors upon the stimulation with foreign pathogens. These proteins are known to have an impact in fine-tuning the immune response during infection diseases (Kusanovic et al., 2010). A form of TREM knowing as soluble TREM-1 (sTREM-1) has been shown to be implicated in the process of intrauterine

infections as well as non-infectious inflammatory conditions (Jiyong et al., 2009). In septic patients, sTREM has been widely used as a biomarker for the diagnosis and prognosis of bacterial infection (Gibot et al., 2007).

Various clinical studies have been conducted to examine the occurrence of an increased concentration of sTREM in cervicovaginal fluid and serum as well as amniotic fluid of patients with spontaneous preterm delivery. A clinical study in 2010 used amniocentesis to examine the correlation between the concentration of sTREM with gestational age, parturition, and intra-amniotic infection and inflammation. The concentration levels of sTEM were determined by enzyme-linked immunoassay (ELISA) on amniotic fluid samples. The study classified 434 patients into four groups, based on time of labor onset and presence of an infection. sTREM-1 protein was discovered in all the amniotic samples collected. A higher median concentration of sTREM-1 was observed in the group of women delivering at term in comparison to those women in the mid-trimester (14-18 weeks). Patients who delivered at preterm with intrauterine infection had significantly higher median concentrations of sTREM-1 in the amniotic fluid compared to those who delivered at preterm without intrauterine infection. Subsequently, women with preterm premature rupture of membranes (PROM) and intrauterine infection showed significantly higher median levels of sTREM-1 in the amniotic fluid samples in comparison to those without intrauterine infection (Kusanovic et al., 2010). In all cases of preterm labor, significant elevated levels of sTREM-1 were present with intrauterine infection, which indicates that sTREM-1 plays a role in the immune response against intrauterine infection during preterm labor and significantly elevated levels of sTREM are indicative of intrauterine infection.

In another study, sTREM-1 concentrations in maternal serum during term and preterm labor were compared between laboring women either at term or preterm. Women who were at-

term-not-in-labor were sampled prior to their Caesarean section. Concentrations of sTREM-1 were determined using an ELISA. In this study, a higher median concentration of sTREM-1was observed in women at-term-in-labor in comparison to those at-term-not-in -labor. However, significantly higher median concentrations of sTREM-1 were observed in women with preterm labor in comparison to women at term in labor (Tency, 2014). These findings are similar to those of the 2010 study, suggesting a direct correlation between elevated levels of sTREM-1 and preterm labor.

Overall, the findings from these studies support the idea that an increase in sTREM-1 concentration levels with gestational age is a normal process. An increase in the concentration level of sTREM-1 with gestational age supports the idea that gestation resembles an immune response at the molecular level. In addition, it can be concluded that there exists a correlation between concentrations of sTREM-1 and intrauterine infection. Patients who delivered preterm with intrauterine infection had significantly higher median concentrations of sTREM-1in the amniotic fluid compared to preterm labor patients without intrauterine infection. These higher median concentrations of sTREM-1 significantly differ from that of term delivery, thus adding further support to the idea that intrauterine induced immune response during preterm labor produces a different assembly of chemical mediators, including a higher than normal concentration of sTREM-1. As a result, concentration levels of sTREM-1 may be a credible biomarker for intrauterine infection in the amniotic cavity.

5.2 Matrix metalloproteinases

Matrix metalloproteinases (MMPS) constitute a multigenic family of proteolytic, zinc-dependent enzymes. They are initially secreted in their inactive form as proenzymes. The primary function of most MMPs is to degrade various proteins of the extracellular matrix (ECM)

and basement membrane of tissue. The degradation of extracellular matrix proteins such as collagens and fibronectins weakens the membranes of the uterus, eventually leading to the rupture of membranes. Substrate specificity is highly diverse amongst MMPs. Some MMPs such as MMP -1, -8, and -13 are collagenases and target fibrillar collagens and non-fibrillar collagens while others such as MMP-2 and -9 are gelatinases, targeting collagen IV, V, elastin, proteoglycan and fibronectin (Goffin, 2003). The various specificities of MMPs enable them to participate in physiological processes such as innate and adaptive immunity, inflammation, angiogenesis, remodeling, and the control of key reproductive events such as ovulation, embryo implantation, and uterine contraction (Amalinei et al., 2007). Endogenous inhibitors termed tissue inhibitors of MMPs (TIMPs) regulate the activity of MMPs. Brew (2010), To date four TIMPs have been identified: TMP -1,-2, -3, and -4. (Stetler-Stevenson,1992; Wang, 1996). An imbalance between the concentration of MMPs and TIMPs has the potential to result in various medical conditions such as tumor invasion, rheumatoid arthritis, atherosclerosis, and aneurysms (Amalinei et al., 2007).

Human pregnancy is the steady remodeling of the collagenous extracellular matrix. Matrix metalloproteinases are involved in this process through membrane weakening and the rupture and cervical ripening and dilation (Vadillo-Ortega and Estrada-Gutiérrez, 2004). Certain MMPs such as MMP-1, -2, and -3 are consistently expressed during the entirety of the gestation cycle, while others such as MMP-9 are produced as a result of active labor. Various studies have sought to examine the difference in the concentration levels of MMPs, TIMPs, and MMP: TIMP ratios in the amniotic fluid during various stages of the gestation cycle in both women delivering at term and preterm (Tency, 2014; Vadillo-Ortega and Estrada-Gutiérrez, 2004; Epstein, 2009)

The production of an elevated level in MMP expression may be the consequence of intrauterine infection during gestation and thus a biomarker for intrauterine infection. This hypothesis was further developed by stating that an elevated level in the production of MMPs has the potential to cause aberrant extracellular matrix degradation of the chorioamnion and cervix which results in preterm delivery. Thus, this study sought to determine maternal serum concentrations of MMP -3, -9, and all four TIMPs along with the MMP:TIMP ratios during term and preterm labor (Tency, 2014).

The results indicated that levels of MMP-9 are elevated in the maternal serum during preterm labor. However, no changes in the concentration of MMP-3 in the maternal serum relating to either term or preterm parturition were observed. In addition, TIMP expression in the maternal serum revealed a progressive increase in concentration of both TIMP-1 and -2 with advancing gestation age irrespective of labor status. The most valuable piece of information gathered was the observation of an elevated level in both the MMP-9:TIMP-1 and MMP-9:TIMP-2 ratios in women with preterm labor (Tency, 2014). Although further studies are needed to verify this increase in the ratios of MMP-9:TIMP-1 and MMP-9:TIMP-2 in correlation with matrix degradation, there are a small number of studies that have successfully demonstrated such a correlation. These studies found an increase in concentration of as well as an imbalance between MMPs and TIMPs (i.e. MMP -2, -9, TIMP-2, -1) in the amniotic fluid during PPROM (Vadillo-Ortega and Estrada-Gutiérrez, 2004; Tency, 2014; Epstein et al., 2000).

Studies such as those conducted by Tency show that as a consequence of intrauterine infection, either abnormal concentrations of MMPs or imbalance in the ratio of MMP:TIMP are found to have a direct link in the pathogenesis of preterm labor. Subsequently, the production of excessive MMPs can lead to irregular extracellular matrix degradation, influence of premature

membrane rupture and cervix ripening (Epstein et al., 2000). Extracellular matrix homeostasis is important in maintaining the tensile strength of the amniochorion. This tensile strength enables the membranes of the amniochorion to perform as a physical and functional boundary for the fetus during gestation. Thus, because of the direct correlation between the production of excessive MMPs and aberrant extracellular matrix degradation, MMPs and the MMP:TIMP ratio as measured in maternal serum presents as a credible biomarker for an increased risk of preterm labor

5.3 Cytokines and chemokines

Cytokines and chemokines are signaling molecules that play a role in cell communication in immune responses by enabling the movement of various cells towards the site of infection and inflammation. The term "cytokines" encompasses various soluble proteins including interleukins (IL), interferons (IFNs), tumor necrosis factor (TNF), and chemokines. Cytokines are involved in the normal development process of a fetus. In order for a successful delivery of a newborn to occur, the developing fetus must avoid causing a maternal immune response, while the maternal immune response must avoid recognizing the fetus as foreign or a threat (McAdams and Juul, 2012). However, in order to mount a defense against a bacterial infection, the maternal system must be activated. During intrauterine infections, the concentration levels of cytokines show a significant difference in their expression, which can ultimately influence the onset of parturition and result in preterm labor (Arababadi et al., 2012),.

Over 50 different cytokines are involved in the process of preterm labor and PPROM. Although their specific roles in the onset of preterm labor are still a major focus of current research, it has been shown that significant changes in concentration levels of cytokines such as IL-1, IL-6, IL-10, IL-8, and TNF have a role in the induction of preterm labor (Farina and

Winkelman, 2010). Several studies have shown a change in cytokine production and the subsequent synthesis and release of prostaglandins, which as discussed below, are correlated to induction of preterm labor and PROM.

In one of the earlier studies, conducted in the late 1980's, a direct correlation between an increased production level of IL-1 and the onset of premature parturition was demonstrated. By assaying amniotic fluid of 182 patients, it was found that intrauterine infection was associated with significant IL-1 activity in the amniotic fluid (Romero et al., 1990). These results are further supported by subsequent studies analyzing amniotic samples from patients with intact membranes that were in preterm labor at or before 34 weeks of gestation. Collectively, these studies found that most women had elevated levels of IL-1 alpha and IL-1 beta in their amniotic samples. IL-1 alpha and IL-1 beta are common proinflammatory cytokines that are produced by activated mast cells and macrophages. Specifically, IL-1 beta is an influential inflammation mediator that mediates the expression of prostaglandins by the amnion and decidua. Hence, results obtained from above studies support the presence of subclinical intrauterine infection in preterm deliveries.

In a study done in 1990, levels of interleukin-6 in amniotic fluid were analyzed. The work found elevated levels of IL-6 in amniotic fluid from women in preterm labor with intraamniotic infection (Romero et al., 1990). Furthermore, a study on maternal serum samples, revealed similar results, in regards to IL-6 levels in women in preterm labor with intrauterine infection. In this study, serum samples from the peripheral blood of 60 mothers who delivered at preterm as well as 100 mothers who delivered at term was examined to determine the levels of IL-12, IL-10, and IL-6. Protein concentration levels measured from serum samples showed a

significant increase in the concentration levels of IL-6 and IL-12 in preterm mothers in comparison to mothers who delivered at term (Arababadi et al., 2012).

Additionally, concentration levels of IL-12 and IL-18 have been studied excessively in order to understand the process of preterm labor, specifically the process of intrauterine infection influenced preterm labor. IL-12 and IL-18 are important in the regulation process of natural killer cells during early stages of gestation (Ekman-Ordeberg, 2012). It has been demonstrated that an increase in IL-12 levels in mid-pregnancy is associated with preterm delivery and chorioamnionitis prior to 35 weeks of delivery (Gargano et al., 2008).

In the same study that identified an increased level of IL-6 in maternal serum, it was also found that concentration levels of IL-10 were not significantly different in the two groups (preterm and term). However, despite this report, levels of IL-10 are still a focus of intense study as a possible biomarker of intrauterine infection. IL- 10 decreases the production of pro-inflammatory cytokines such as IL-8, IL-6, TNF-α and prostaglandins (Ekman-Ordeberg, 2012). Normally with the advancement of the gestation cycle, the concentrations of IL-10 decreases in cervical secretions. In a study examining the placental tissues in chorioamnionitis-associated preterm labor and term labor, a significant reduction in the concentration of IL-10 was found in the preterm labor samples when compared with second-trimester normal pregnancy samples (Hannan et al., 2006). Additionally, patients that delivered at preterm without intrauterine infection showed significantly higher concentrations of IL-10 compared to those that delivered at term (Gotsch et al., 2008). Given this conflicting data on IL-10 concentration levels, further research is needed to fully understand the nature of IL-10 in the presence of intrauterine infection and its action on other cytokines.

Though there are many studies reporting changes in concentration of various cytokines during intrauterine infection associated with preterm labor, it is also known that non-infected cervical ripening and labor are also associated with increased cytokine concentrations (Törnblom et al., 2005). Levels of IL-8, IL-6, and MCP-1 were analyzed in cervical biopsies obtained from 50 pregnant women without clinical signs of intrauterine infection at preterm and term pregnancies. While concentration levels of these proteins increased during labor compared to non-labor groups, measurements from these biopsies did not reveal any changes in the protein concentrations of IL-8, IL-6, and MCP-1 between preterm and term deliveries (Törnblom et al., 2005). Studies such as these, that find no significant changes in the concentration levels of certain cytokines in preterm labor when compared to term labor are important because these studies help distinguish between preterm deliveries with intrauterine infection and preterm deliveries without intrauterine infection. Thus, cytokines such as IL-1, IL-6, IL-8, IL-12 and TNF-α are possible biomarkers of intrauterine infection induced preterm labor.

5.4 Prostaglandins

During gestation, prostaglandins are produced in the human placenta, fetal membranes and decidua. Prostaglandins are a group of lipid molecules that play a role in the immune response by prompting an inflammatory response. Their involvement in the immune response at the cellular level is characterized by the gene expression and protein localization. The production of prostaglandins in the body initiates from arachidonic acid through the activity of the cyclooxygenase enzyme complex (Farina and Winkelman, 2010). As pleiotropic mediators, prostaglandins play a significant role in the normal process of parturition.

The inflammatory response is induced by the strategic placement of the choriodecidua. The juxtaposition of the chorion and maternal placenta creates conditions for fetal antigenic

exposure to the maternal immune system (Haddad et al., 2006) and allows for the expression of certain activators that enable the tissues involved in parturition to both synthesize and degrade prostaglandins (Phillips et al., 2014). Prostaglandins are involved in myometrial contractility and regulation of extracellular matrix metabolism changes related to cervical ripening at the onset of labor (Challis et al., 2002).

In addition to playing a role in the normal process of parturition, prostaglandins also are involved in intrauterine infection related preterm labor. The role of prostaglandins in intrauterine infection related preterm labor is studied by comparing amniotic fluid metabolomics and placental tissue profiles of women delivering preterm (in the presence and absence of intra-amniotic infection and inflammation) with those delivering at term (Romero et al., 2003). The concentration of inflammatory proteins within the amniotic fluid is measured to confirm the histological observation of inflammation (Phillips et al., 2014). The presence of an increased concentration of stimulatory prostaglandins by intrauterine tissues is an indication of inflammation induced by intrauterine infection. This increase in the production of stimulatory prostaglandins is generally considered a central component of the cascade of events leading to preterm parturition (Ivanišević et al., 2001).

Prostaglandins are not always activated in the body. The nicotinamide adenine dinucleotide-1 dependent 15-hydroxy prostaglandin dehydrogenase (PGDH) catalyzes the conversion of prostaglandins to the biologically inactive form of 15-keto derivatives (Agrawal and Hirsch, 2012). In bacterial induced preterm labor, the expression of this PGDH molecule has been shown to be down-regulated. Specifically, PGDH activity in chorion membrane is significantly lower in preterm labor than at term (Phillips et al., 2014). The significant low PGDH activity during preterm labor supports the idea that intrauterine infection stimulates the

production of prostaglandins in spite of the absence of PGDH activity, which may lead to the pathogenesis of preterm labor.

The influence of chorioamnionitis on prostaglandin production has also been studied. Placental and fetal membrane tissues samples from 12 women that delivered prematurely between 24 and 35 weeks were compared to placental and fetal membrane tissue samples of 15 women who had normal deliveries at term. The study revealed an association between chorioamnionitis and a significant increase in amniotic prostaglandin production. In the absence of chorioamnionitis the production of prostaglandin by amnions from spontaneous preterm labor was significantly lower in comparison to after spontaneous labor at term (Bernal et al., 1987). Another key piece of information that this study presented was the difference in the concentration levels of prostaglandins towards the completion of term parturition in comparison to concentration levels in the presence of chorioamnionitis. Although prostaglandin synthesis capacity towards the end of term parturition increased, in the presence of chorioamnionitis there was a 30-fold overall increase in amniotic prostaglandin production during preterm parturition (Bernal et al., 1987). Normally, prostaglandins produced in the amnion are inactivated by prostaglandin dehydrogenase, which prevents them from reaching the myometrium and activating uterine contractions. However, in the presence of intrauterine infection, the activity of prostaglandin dehydrogenase is inhibited. This is detrimental, because the production of prostaglandins results in the synthesis and release of matrix metalloproteases (MMPs) (Romero et al., 2002). Independent studies established that the production of abnormal concentrations of MMPs is produced through inflammatory mediators as a consequence of intrauterine infection (Goldenberg et al., 2008; Tency, 2014). Subsequently, the production of excessive active prostaglandins can lead to increased levels of MMPs, which in turn can lead to aberrant

extracellular matrix degradation, premature membrane rupture and cervix ripening (Romero et al., 2002).

In addition, various other studies reveal higher amniotic fluid levels of prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PG\ F_{2\alpha}$) production in women with preterm labor and microbial invasion of the amniotic cavity (Monga and Blanco, 1995). Primary prostaglandins such as PGE_2 and PGF_2 are produced from the precursor arachidonic acid (AA). Membrane phospholipids, such as phosphatidyl ethanolamine and phosphatidyl inositol, release these primary prostaglandins through the action of one or more isozymes of phospholipase C or phospholipase A_2. Phospholipase A_2 consists of a superfamily of enzymes that catalyzes the hydrolysis of the phospholipid ester bond, which generates a free fatty acid and a lysophospholipid. This Phospholipase A_2 pathway is the primary pathway in which AA is released from phospholipids (Balsinde et al., 2002). Subsequently, the prokaryotic phospholipase A_2 domain is found in bacterial and fundal phospholipases. To better understand the mechanism of primary prostaglandin up-regulation in women with intrauterine infection, the role of phospholipase A_2 activity from the microorganisms colonizing endocervical and/or uterus was studied (Han et al., 2004). With the correlation between phospholipase A_2 and AA mentioned above, the increase in phospholipase A_2 activity may lead to an increased production of AA from amniotic phospholipids. An increase in the concentration levels of AA eventually results in the production of increased prostaglandin synthesis, which is postulated to trigger early parturition (Bennett et al., 1987).

Ultimately, an increase in uterine contractility during preterm labor is the result of activation and stimulation of the myometrium. The stimulation of the myometrium occurs through increased concentrations of prostaglandins and oxytocin. Increased production of

prostaglandins by intrauterine tissues is assumed to play a central role during the cascade of events leading to preterm parturition. Prostaglandins mediate cervical ripening and stimulate uterine contractions and indirectly increase fundal-dominant myometrial contractility and synchronization by up regulation of gap junctions, oxytocin, and arginine vasopressin receptors (Ivanišević et al., 2001).

Researchers have noted during the pathophysiology of infection-associated preterm labor, elevated levels of proinflammatory cytokine-prostaglandins are produced. Various studies have revealed an increase in proinflammatory cytokines including IL-1, IL-6, IL-10, IL-8, and TNF (Behrman et al., 2007; Agrawal et al., 2012; Goldenberg et al., 2008). An increase in such cytokines stimulates the production of prostaglandins and initiates neutrophil chemotaxis, infiltration, and activation (Romero et al., 2002). This is consistent with a number of studies that have established a relationship between chorioamnionitis and an increase in amniotic prostaglandin production together with an increased concentration of soluble triggering receptor expressed on myeloid cells (sTREM). Subsequently, the upregulation of sTREM is directly correlated with the upregulation of cytokines as a result of the role maintained by sTREM during an immune response. TREM are mainly expressed on monocytes and neutrophils as cell surface receptors upon the stimulation with foreign pathogens. These proteins are known to have an impact in fine-tuning the immune response during infection diseases (Kusanovic et al., 2010).

Despite these observed relationships amongst the increased concentrations of proinflammatory mediators under the presence of intrauterine infection, a concrete list of biomarkers for preterm labor is yet to be established. The nature of these proinflammatory mediators varies from patient to patient, thus it is difficult to say that for example, an elevated concentration of cytokine IL-6 guarantees the presence of intrauterine infection. As a result, the

identification of biomarkers of intrauterine infection is a still a major focus of the research conducted today.

6. Treatments to prevent preterm labor

Preterm labor contributes to over 70% of perinatal mortality in developed countries. In comparison to infants born at term, newborns that survive preterm labor are likely to suffer cardiorespiratory problems, mental retardation, cerebral palsy, and vision and hearing impairment (Flood and Malone, 2012). Although preterm labor is the consequence of numerous pathways with individual etiologies and initiators, the pathophysiology of infection-associated preterm labor is thought to be a causative factor of preterm labor.

Various treatment methods are used during preterm labor to help women stay pregnant longer. One method includes the administration of antenatal corticosteroids (ACS) to increase the rate of the infant's lung development. Tocolytics are used to delay labor for a couple of days, by slowing or stopping contractions to provide pregnant women enough time to obtain ACS or to get to a hospital with a neonatal intensive care unit (March of Dimes Foundation, 2014). Despite these treatment methods, there is no treatment to stop preterm labor as a whole process. However, there have been proposed treatment mechanisms directed towards specific biological pathways that are thought to lead to preterm labor, including the intrauterine infection and inflammation biological pathway.

6.1 Treatment of bacterial vaginosis

Bacterial vaginosis is characterized as a reduction of normal lactobacillary bacteria with a heavy overgrowth of mixed anaerobic flora including *Gardnerella vaginalis*, *Mycoplasma hominis* and *Mobiluncus* species (Hay, 2002). Bacterial vaginosis may spontaneously resolve

without treatment. However, as a consequence of its asymptomatic nature, most women who develop bacterial vaginosis in early pregnancy are likely to have persistent infection later in their pregnancy. Chorioamnionitis, the presence of active infection in the amniotic sac, is a polymicrobial process. Notably, the development of bacterial vaginosis in early pregnancy may colonize the amniotic fluid with low virulence. Although, it is still unclear what mechanisms enable these bacteria to transition from low virulent colonizers to pathologic contributors leading to chorioamnionitis (Johnson et al., 2014). With the correlation between intrauterine infection and preterm labor, the opportunity exists in treating bacterial vaginosis before it advances to the uterus in order to reduce the preterm birth rate (Hay, 2002).

In an early study, randomized trials were set up to assess the effects of antibiotic treatment of bacterial vaginosis in pregnancy. Women from various age groups under multiple stages of pregnancy that were both symptomatic and asymptomatic to bacterial vaginosis were administered antibiotic regimens. From the 5888 women that participated in the study, antibiotic therapy was shown to be effective at eradicating bacterial vaginosis during pregnancy prior to its pathological state in 80%. It was found that treatment of bacterial vaginosis prior to 20 weeks of gestation may reduce the risk of extreme preterm birth, less than 37 weeks. However, they were unable to detect a reduction in the risk for preterm labor or preterm rupture of membranes (PROM) with the administration of the antibiotic regimens near 37 weeks of gestation. In addition to these finding, it was also shown that administration of antibiotic regimens did not affect the risk of subsequent preterm labor in women who had experienced a previous preterm labor delivery (McDonald et al. 1994).

Subsequently, a study conducted in Germany sought to prevent preterm labor by pH-self measurement. Women used a self-care program to measure vaginal pH by means of test gloves

twice a week. The objective was for the women to self-monitor for the goal of early detection of any abnormality in their vaginal milieu. If abnormal pH (pH ≥ 4.7) or other risk factors were present, they were given the option of undergoing *lactobacillus acidophilus* therapy or the administration of vaginal clindamycin (a class of medications called lincomycin antibiotics specifically used to treat bacterial vaginosis). Those women who refused any treatment served as a control group for the research. The prematurity rate in the self-measurement/intervention group was 8.1% versus 12.3% in the control group. As a result of these finding, starting 1 March 2000, the pH screening program was recommended to pregnant women in order to reduce prematurity. The expectation was that, there would be a significant decrease of prematurity for the second half of 2000. In the study area there was an overall decrease of prematurity from 7.68 to 6.81% and a reduction of cases of preterm delivery at ≤ 32 weeks from 3.22 to 2.39% (Hoyme and Saling, 2004). Results such as these support the idea that early detection and then treatment of bacterial vaginosis can impact the incidence of preterm labor.

6.2 Treatment of chorioamnioitis

Chorioamnionitis is a global disease and the diagnosis of chorioamnionitis varies widely across different institutions and countries. The clinical diagnosis of chorioamnionitis includes the presence of intrapartum fever (fever greater than 100.4 °F), uterine tenderness, maternal tachycardia, fetal tachycardia, and purulent amniotic fluid (Johnson et al., 2014). Although such clinical measures are widely used as a basis to administer antibiotics to improve the outcomes of chorioamnionitis, they are still perceived as unreliable clinical symptoms of the disease (e.g. fever greater than 100.4°F could indicate the existence of numerous other health issues aside from chorioamnionitis). Amniotic fluid sampling and culture along with histopathological diagnosis provide a concrete standard for diagnosis of chorioamnionitis. Unfortunately, as a

consequence of the invasive nature of these procedures, data from them are usually not obtained until after delivery.

Various studies have been conducted to determine whether the administration of certain antibiotic regimens can improve maternal symptoms and decrease incidence of adverse perinatal outcomes. Antibiotic regimens are studied by comparing one antibiotic regimen targeted against bacterial vaginosis and chorioamnionitis with placebo or no treatment (Seelbach-Goebel, 2013; Mercer, 2012). Randomized trials comparing antibiotic therapy with placebo in PROM or preterm labor of 34 weeks or less were used to determine whether antibiotics prolonged pregnancy and reduced neonatal morbidity in PROM and preterm labor. This use of trials led to the discovery that antibiotics were associated with prolongation of pregnancy in PROM together with a reduction in neonatal morbidity in women with PROM at gestation of 34 weeks or less. However, they found no such benefit in preterm labor with the administration of antibiotics (Hutzal et al. 2008).

Further studies that have strived to discover whether antibiotic administration improved neonatal and maternal outcomes in women with preterm labor found no such correlation. A broad-spectrum antibiotic regime was tested in patients with preterm labor and intact membranes with the goal of improvement in neonatal and maternal outcomes, particularly in patients with microbial invasion of the amniotic cavity (Ovalle et al. 2006). Of the thirty-nine women who received the combination of antibiotics, no improvement in maternal or perinatal outcome in patients with preterm labor and intact membranes was identified. Therefore the current practice is to administer antibiotic treatment when patients have been diagnosed with clinical chorioamnionitis rather than treat patients that are at risk for, but do not exhibit any symptoms of

chorioamnionitis or preterm labor. Thus, the necessity of discovering a therapeutic regimen for acute impending preterm delivery due to chorioamnionitis remains.

7. Conclusion

Various research efforts in both clinical settings and laboratory settings have been focused on understanding the process of preterm labor. One promising area of research is the effort of identifying inflammatory markers of preterm labor in asymptomatic and symptomatic women. The ideal site for the identification of inflammatory markers is the amniotic fluid. Although the performance of an amniocentesis is possible, it is considered an invasive procedure posing potential risks. These potential risks include the risk of miscarriage due to rupture of membranes of the amniotic sac and the induction of infection in the uterus. Thus, more often non-invasive procedures such as the analysis of maternal blood, vaginal or cervical secretions are performed to determine the presence of inflammatory markers of intrauterine infection

Determining credible biomarkers of intrauterine infection and chorioamnionitis is necessary to develop effective treatment methods against preterm labor. Despite current studies that identify an increase in sTREM-1, MMPs, cytokines, and prostaglandins during intrauterine infection and chorioamnionitis, it is important to note the variability in the assessment criteria for the diagnosis of histological chorioamnionitis. This variability in the assessment criteria influences the interpretation of results from studies that are conducted on both histological chorioamnionitis and preterm delivery. Currently, there are various antibiotic regimens that have shown significant improvement in preventing the progression of bacterial vaginosis in pregnant women. However, these regimens are not effective in treating the pathological state of vaginosis, intrauterine infection and chorioamnionitis (Ovalle et al., 2006; Hutzal et al., 2008). Thus, further study is necessary to conclude a precise list of credible biomarkers to develop effective

treatment methods against preterm labor. However, with the current research and understanding on the heterologous etiology of preterm labor, the outlook for the development of an effective treatment method is promising. Using reliable biomarkers in conjunction with targeted therapy for known contributors to preterm labor, may result in a decrease in the occurrence of preterm labor, PROM, and increase the health of newborns.

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